



## Assay Development Report

### Theranos Systems Multiplexed Human IL-6, Human TNF- $\alpha$ , Human CRP (hs)

---

#### Contents

1. Introduction
2. Storage and Use
3. Calibration
4. Range
5. Quantitation Limits and Accuracy
6. Precision
7. Specificity
8. Linearity
9. Matrix Effects
10. Stability

#### 1. Introduction

The Theranos Assay System is a fully automated means for measuring concentrations of analytes (biomarkers, drugs) using immunoassay methodology. The system is comprised of instruments, single-use cartridges and a wireless communications link that conveys protocol information to the instruments from a Theranos Server and relays assay data to the Server for interpretation and distribution. Blood, plasma serum and control materials may be analyzed by the System. Calibration is performed at Theranos on a cartridge-lot-specific basis.

The System accepts a metered sample (25uL) from a proprietary sampling device or a pipette, dilutes it automatically to levels appropriate to each assay then executes an automated ELISA assay protocol. The protocol is selected from a set of released protocols available on the Theranos Server and identified by reading a bar code on each cartridge. The bar code is also linked to an assay lot-specific calibration algorithm. Assays are complete in about one hour.

Assays are typically grouped (multiplexed) in particular cartridges designed to monitor specific disease and therapeutic processes. For example, a cartridge designed to monitor acute and inflammatory processes measures IL-6, TNF- $\alpha$  and CRP. Customer is interested in use of the Theranos System and has sponsored a validation exercise at Theranos focused on the inflammatory marker cartridge.

In this exercise, many instruments (60) and three lots of cartridges were used.

#### 2. Storage and Use

Theranos cartridges should be stored in the original unopened packaging in an upright position at 4°C. Theranos instruments require no user maintenance or calibration. User prompts are provided on a screen which is part of the instrument.

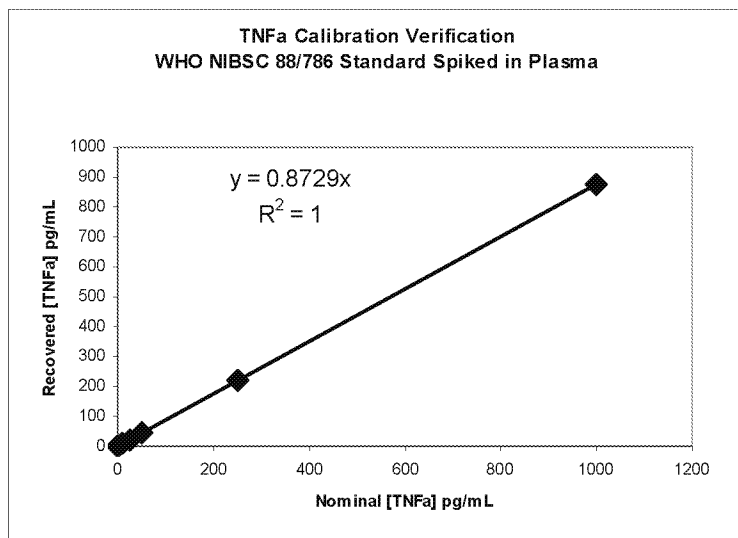
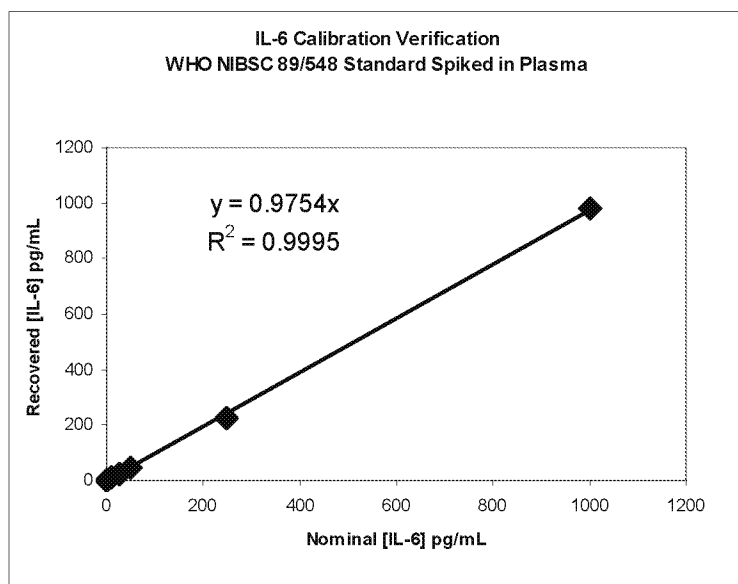


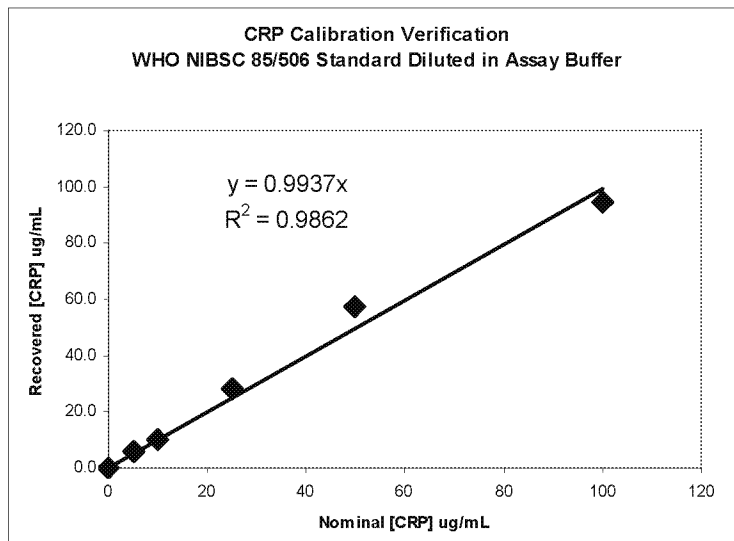
### 3. Calibration

IL-6 and TNF- $\alpha$  assay calibration utilize recombinant analytes expressed in human-cell lines as calibration materials. These are reportedly more stable than recombinant analytes made in bacteria and more similar to the naturally occurring analytes. The CRP assay is calibrated with a human plasma-derived analyte. Theranos Systems assays recognize “natural”, recombinant, and human-cell line expressed recombinant forms of IL-6 and TNF- $\alpha$ . Each lot of Theranos Cartridges is individually calibrated, the calibration equation is linked to the cartridge barcode and results are automatically computed on the Theranos data server. For this validation study, three cartridge lots were produced and calibrated.

#### NIBSC WHO Verification of Calibration

Exemplary assay responses are shown in Appendix A. Calibrations for IL-6, TNF- $\alpha$  and CRP were verified by testing the recovery of the current National Institute for Biological Standards and Control (NIBSC) World Health Organization (WHO) Reference Standards. The current WHO standard for IL-6 is NIBSC code 89/548 (recombinant protein produced in CHO cells with post translational modifications), for TNF- $\alpha$  NIBSC code 88/786 (a natural human protein derived from human BALL-1 cells), and for CRP NIBSC code 85/506 from human plasma. Spike recovery of all three WHO standards were within acceptable limits across the assay ranges as shown in the figures and tables below. Note that for the TNF- $\alpha$  assay we found low recovery (about 30%) of the WHO standard in a reference kit (R&D Systems Quantikine HS catalogue # HSTA00D, data shown in Appendix B). Therefore comparisons of sensitivity and slopes of assay correlations of results of the Theranos System with those of R&D Systems kits will show different results due to their respective calibrations. For example, the R&D Systems Assay would report a TNF- $\alpha$  value of 4 pg/mL when the Theranos Assay reports 12 pg/mL. If desired by a customer the Theranos System can be configured (in calibration algorithms) to provide results matching those of R&D Systems assays (or those of other predicate assay). It is our intention however to continue to perform primary calibration of Theranos assays using International Standard materials whenever possible since predicate assays not so calibrated may be subject to lot-to-lot variation in calibration.





**Theranos Systems Recovery of IL-6 (NIBSC code 89/548) Spiked in Plasma**  
n=3 cartridges, 3 instruments per level

| [IL-6]<br>IU/mL | [IL-6]<br>pg/ml | Recovered<br>[IL-6] pg/mL | CV % | Minus<br>Endogenous | % Recovery |
|-----------------|-----------------|---------------------------|------|---------------------|------------|
| 100             | 1000            | 981.1                     | 11   | 980.1               | 98         |
| 25              | 250             | 227.1                     | 16   | 226.2               | 90         |
| 5               | 50              | 45.2                      | 10   | 44.2                | 88         |
| 3               | 25              | 21.5                      | 8    | 20.5                | 82         |
| 1               | 10              | 10.5                      | 9    | 9.5                 | 95         |
| 0               | 0               | 1.0                       | 47   | 0.0                 | N/A        |

**Theranos Systems Recovery of TNF- $\alpha$  (NIBSC code 88/786) Spiked in Plasma**  
n=3 cartridges, 3 instruments per level

| [TNF $\alpha$ ]<br>IU/mL | [TNF $\alpha$ ]<br>pg/mL | Recovered<br>[TNF- $\alpha$ ] pg/mL | CV % | Minus<br>Endogenous | % Recovery |
|--------------------------|--------------------------|-------------------------------------|------|---------------------|------------|
| 46.5                     | 1000                     | 873.4                               | 3    | 873.0               | 89         |
| 11.6                     | 250                      | 218.7                               | 3    | 218.3               | 96         |
| 2.3                      | 50                       | 44.0                                | 10   | 43.5                | 96         |
| 1.2                      | 25                       | 20.9                                | 22   | 20.4                | 95         |
| 0.5                      | 10                       | 10.9                                | 19   | 10.5                | 100        |
| 0                        | 0                        | 0.4                                 | 14   | 0.0                 | N/A        |

**Theranos Systems Recovery of CRP (NIBSC code 85/506) in Assay Buffer**  
n=3 cartridges, 3 instruments per level

| [CRP]<br>IU/mL | [CRP]<br>ug/ml | Recovered<br>[CRP] ug/mL | CV % | % Recovery |
|----------------|----------------|--------------------------|------|------------|
| 98             | 100            | 94.6                     | 2    | 95         |
| 49             | 50             | 57.4                     | 18   | 115        |
| 24.5           | 25             | 28.1                     | 15   | 113        |
| 10             | 10             | 10.2                     | 14   | 102        |
| 4.9            | 5              | 5.7                      | 20   | 114        |



|   |   |     |    |     |
|---|---|-----|----|-----|
| 0 | 0 | 0.0 | 30 | N/A |
|---|---|-----|----|-----|

#### 4. Range

Reportable ranges based on calibration to WHO standards determined for these assays are:

| Assay         | Low                  | High       |
|---------------|----------------------|------------|
| IL-6          | 2 pg/mL              | 1000 pg/mL |
| TNF- $\alpha$ | 4 <sup>1</sup> pg/mL | 1000 pg/mL |
| CRP           | 0.05 ug/mL           | 100 ug/mL  |

As shown below, all three tested lots support these ranges<sup>2</sup>.

#### 5. Quantitation Limits

Assay calibrations and determination of Lower Limit of Quantitation (LLOQ) and Upper Limit of Quantitation (ULOQ) were performed and analyzed by proprietary software. Assay responses were fitted by a four-parameter equation and LLOQ and ULOQ determined according to FDA criteria. Calibrators were run in triplicate on three days (consecutive or non-consecutive) on 36 instruments for a total of nine cartridges per level, at 12 levels.

##### Summary of Calibration Analysis for three Cartridge Lots

| Lot 2455142005 | IL-6       | TNF- $\alpha$ | CRP        |
|----------------|------------|---------------|------------|
| LLOQ           | 1.3 pg/mL  | 6.3 pg/mL     | 0.05 ug/mL |
| ULOQ           | 1000 pg/mL | 1000 pg/mL    | 150 ug/mL  |
| Lot 2455146006 | IL-6       | TNF- $\alpha$ | CRP        |
| LLOQ           | 1.3 pg/mL  | 6.3 pg/mL     | 0.05 ug/mL |
| ULOQ           | 1000 pg/mL | 1000 pg/mL    | 150 ug/mL  |
| Lot 2455156002 | IL-6       | TNF- $\alpha$ | CRP        |
| LLOQ           | 2.3 pg/mL  | 6.3 pg/mL     | 0.05 ug/mL |
| ULOQ           | 1000 pg/mL | 1000 pg/mL    | 150 ug/mL  |

#### Limits of detection (LOD)

The range in the Limits of detection calculated as  $2 \times \text{Signal SD} / \text{Slope of dose response}$  ( $\square \text{signal} / \square \text{conc}$ ) are reported for the three lots of Theranos cartridges. Comparison data are also given for R&D Systems assays Minimum Detectable Dose “MDD” (which is equivalent to LOD). In addition to the calibration issue for the R&D Systems TNF- $\alpha$  assay discussed above which gives a four-fold lower limit for R&D Systems, we believe the calculation of MDD performed by R&D Systems may be compromised (falsely low) by the inability of any known

<sup>1</sup> Equivalent to 1 pg/mL in the R&D Systems assay calibrated using R&D Systems calibrators

<sup>2</sup> The lower limit of the reportable range of the TNF- $\alpha$  assay has been extended below the LLOQ so as not to restrict the reportable range too much. The LLOQ is higher than anticipated due to unexpectedly high imprecision of the assay in the cartridge lots used for validation compared with other cartridge lots used in pre-clinical work. We are presently investigating the root cause of this imprecision.



spectrometer to report optical density to the required precision needed to support the calculated values.

The CRP MDD reported by R&D Systems is highly misleading since it represents the concentration in the assay rather than in the sample (which “must be diluted” according to their package insert prior to assay). Note that the Theranos assay uses a sample which is diluted 5000-fold. If we compare the actual sensitivity *in the assay medium* the Theranos value would be about 0.006 ng/mL.

| Assay System             | IL-6 (pg/mL) | TNF- $\alpha$ (pg/mL) | CRP (ng/mL)  |
|--------------------------|--------------|-----------------------|--------------|
| Theranos                 | 0.9 – 1.5    | 3.7 – 5.2             | 28 - 31      |
| R&D Systems              | 0.02 – 0.11  | 0.04 – 0.19           | 0.005 – 0.22 |
| R&D Systems <sup>3</sup> |              | 0.16 – 0.76           |              |

## 6. Precision and Accuracy

Plasma with low endogenous analyte levels was spiked with three levels of the analytes were measured in 16 cartridges per level on 48 instruments. Recovery of the spiked analyte was good. Imprecision (% CV) ranged from 10 - 25 %. Note that the imprecision cited includes both instrument-instrument and cartridge-cartridge variance.

### Spiked Plasma Samples (n=16 cartridges, n=48 instruments)

| Nominal [IL-6] pg/mL | Recovered [IL-6] pg/mL | StDev | CV % | % Recovery |
|----------------------|------------------------|-------|------|------------|
| 800.3                | 806.9                  | 79.8  | 9.9  | 101        |
| 50.3                 | 50.5                   | 4.7   | 9.2  | 100        |
| 5.3                  | 5.1                    | 0.8   | 15.5 | 96         |
| Nominal [TNFa] pg/mL | Recovered [TNFa] pg/mL | StDev | CV % | % Recovery |
| 500.3                | 418.9                  | 39.6  | 9.5  | 84         |
| 50.3                 | 42.7                   | 5.1   | 12.0 | 85         |
| 12.3                 | 12.9                   | 3.2   | 24.6 | 105        |
| Nominal [CRP] ug/mL  | Recovered [CRP] ug/mL  | StDev | CV % | % Recovery |
| 50.1                 | 50.4                   | 10.0  | 19.9 | 101        |
| 1.6                  | 1.6                    | 0.3   | 16.8 | 97         |
| 0.1                  | 0.1                    | 0.0   | 20.6 | 103        |

## 7. Specificity

Assays were tested for cross reactivity and interference by the factors listed below, at high, mid and low analyte levels. Potential cross-reactants were selected based on package inserts of recognized predicate methods and added at levels deemed to be higher than those likely to be found in clinical samples. No significant cross reactivity or interference was observed for any of the assays by any of the tested factors at all analyte levels tested.

<sup>3</sup> Recalculated to reflect calibration to WHO standard material



| <b>IL-6 Assay Specificity Test in Spiked Plasma (n=3 cartridges, 3 instruments per level)</b> |                                   |                                |                                   |             |                   |
|---|-----------------------------------|--------------------------------|-----------------------------------|-------------|-------------------|
| <b>Substance</b>  | <b>[Test Substance]<br/>ng/mL</b> | <b>Target<br/>[IL-6] pg/mL</b> | <b>Recovered<br/>[IL-6] pg/mL</b> | <b>CV %</b> | <b>% Recovery</b> |
| Control   | 0                                 | 1000.3                         | 1100.3                            | 7.8         | 110               |
|   | 0                                 | 90.3                           | 95.8                              | 16.6        | 106               |
|   | 0                                 | 8.3                            | 9.4                               | 4.8         | 113               |
| IL-1 $\alpha$   | 10                                | 1000.3                         | 939.2                             | 2.9         | 94                |
|   | 10                                | 90.3                           | 97.0                              | 15.7        | 107               |
|   | 10                                | 8.3                            | 9.0                               | 6.9         | 108               |
| IL-2  | 10                                | 1000.3                         | 1047.7                            | 1.7         | 105               |
|   | 10                                | 90.3                           | 86.7                              | 9.4         | 96                |
|   | 10                                | 8.3                            | 8.7                               | 22.3        | 105               |
| IL-3  | 10                                | 1000.3                         | 950.0                             | 12.7        | 95                |
|   | 10                                | 90.3                           | 91.9                              | 4.6         | 102               |
|   | 10                                | 8.3                            | 7.9                               | 4.4         | 95                |
| IL-4  | 10                                | 1000.3                         | 908.0                             | 10.9        | 91                |
|   | 10                                | 90.3                           | 79.9                              | 16.7        | 88                |
|   | 10                                | 8.3                            | 8.1                               | 18.1        | 97                |
| IL-6 sR   | 50                                | 1000.3                         | 914.9                             | 18.0        | 91                |
|   | 50                                | 90.3                           | 81.2                              | 1.3         | 90                |
|   | 50                                | 8.3                            | 8.0                               | 29.0        | 96                |
| IL-7  | 10                                | 1000.3                         | 895.0                             | 10.0        | 89                |
|   | 10                                | 90.3                           | 78.1                              | 9.1         | 87                |
|   | 10                                | 8.3                            | 8.2                               | 9.4         | 99                |
| IL-8  | 10                                | 1000.3                         | 927.8                             | 9.7         | 93                |
|   | 10                                | 90.3                           | 82.3                              | 17.1        | 91                |
|   | 10                                | 8.3                            | 8.4                               | 17.6        | 101               |
| IL-11   | 10                                | 1000.3                         | 897.5                             | 12.5        | 90                |
|   | 10                                | 90.3                           | 90.3                              | 6.1         | 100               |
|   | 10                                | 8.3                            | 7.9                               | 2.2         | 95                |
| IL-12   | 10                                | 1000.3                         | 837.6                             | 8.4         | 84                |
|   | 10                                | 90.3                           | 85.8                              | 14.7        | 95                |
|   | 10                                | 8.3                            | 6.8                               | 18.1        | 82                |
| CNTF  | 10                                | 1000.3                         | 900.6                             | 8.4         | 90                |
|   | 10                                | 90.3                           | 95.3                              | 5.8         | 106               |
|   | 10                                | 8.3                            | 8.9                               | 22.4        | 107               |
| G-CSF   | 10                                | 1000.3                         | 925.0                             | 18.7        | 92                |
|   | 10                                | 90.3                           | 90.2                              | 12.8        | 100               |
|   | 10                                | 8.3                            | 9.7                               | 6.9         | 117               |
| sgp130  | 1000                              | 1000.3                         | 895.5                             | 17.0        | 90                |
|   | 1000                              | 90.3                           | 88.6                              | 2.0         | 98                |
|   | 1000                              | 8.3                            | 9.4                               | 3.2         | 114               |
| LIF R   | 50                                | 1000.3                         | 895.2                             | 2.8         | 89                |
|   | 50                                | 90.3                           | 78.5                              | 16.5        | 87                |
|   | 50                                | 8.3                            | 8.9                               | 19.8        | 107               |
| OSM   | 10                                | 1000.3                         | 945.4                             | 9.5         | 95                |
|   | 10                                | 90.3                           | 77.1                              | 10.0        | 85                |



| <b>IL-6 Assay Specificity Test in Spiked Plasma (n=3 cartridges, 3 instruments per level)</b> |                                   |                                |                                   |             |                   |
|---|-----------------------------------|--------------------------------|-----------------------------------|-------------|-------------------|
| <b>Substance</b>  | <b>[Test Substance]<br/>ng/mL</b> | <b>Target<br/>[IL-6] pg/mL</b> | <b>Recovered<br/>[IL-6] pg/mL</b> | <b>CV %</b> | <b>% Recovery</b> |
|   | 10                                | 8.3                            | 6.9                               | 16.8        | 83                |
| TNF- $\beta$  | 10                                | 1000.3                         | 919.6                             | 8.6         | 92                |
|   | 10                                | 90.3                           | 83.3                              | 15.8        | 92                |
|   | 10                                | 8.3                            | 9.4                               | 7.8         | 113               |
| IL-1 $\beta$  | 10                                | 1000.3                         | 901.2                             | 8.1         | 90                |
|   | 10                                | 90.3                           | 85.7                              | 17.6        | 95                |
|   | 10                                | 8.3                            | 7.5                               | 10.5        | 90                |
| sTNF RI   | 10                                | 1000.3                         | 1025.2                            | 9.2         | 102               |
|   | 10                                | 90.3                           | 83.4                              | 11.4        | 92                |
|   | 10                                | 8.3                            | 9.4                               | 16.5        | 114               |
| sTNF RII  | 10                                | 1000.3                         | 963.3                             | 13.8        | 96                |
|   | 10                                | 90.3                           | 90.7                              | 10.2        | 100               |
|   | 10                                | 8.3                            | 9.3                               | 21.0        | 112               |

| <b>TNF-<math>\alpha</math> Assay Specificity Test in Spiked Plasma (n=3 cartridges, 3 instruments per level)</b> |                                   |                                |                                   |             |                   |
|--|-----------------------------------|--------------------------------|-----------------------------------|-------------|-------------------|
| <b>Substance</b>   | <b>[Test Substance]<br/>ng/mL</b> | <b>Target<br/>[TNFa] pg/mL</b> | <b>Recovered<br/>[TNFa] pg/mL</b> | <b>CV %</b> | <b>% Recovery</b> |
| Control  | 0                                 | 900.3                          | 883.7                             | 4.1         | 98                |
|  | 0                                 | 90.3                           | 85.4                              | 4.1         | 95                |
|  | 0                                 | 8.3                            | 8.3                               | 40.4        | 100               |
| IL-1 $\alpha$  | 10                                | 900.3                          | 849.1                             | 5.5         | 94                |
|  | 10                                | 90.3                           | 89.6                              | 12.7        | 99                |
|  | 10                                | 8.3                            | 8.8                               | 16.0        | 106               |
| IL-2   | 10                                | 900.3                          | 855.2                             | 23.5        | 95                |
|  | 10                                | 90.3                           | 90.8                              | 7.9         | 101               |
|  | 10                                | 8.3                            | 9.6                               | 18.5        | 116               |
| IL-3   | 10                                | 900.3                          | 836.5                             | 23.5        | 93                |
|  | 10                                | 90.3                           | 74.3                              | 5.4         | 82                |
|  | 10                                | 8.3                            | 8.2                               | 29.2        | 98                |
| IL-4   | 10                                | 900.3                          | 884.6                             | 6.9         | 98                |
|  | 10                                | 90.3                           | 89.5                              | 8.5         | 99                |
|  | 10                                | 8.3                            | 7.0                               | 49.3        | 84                |
| IL-6 sR  | 50                                | 900.3                          | 874.0                             | 23.5        | 97                |
|  | 50                                | 90.3                           | 77.8                              | 13.8        | 86                |
|  | 50                                | 8.3                            | 8.6                               | 34.8        | 103               |
| IL-7   | 10                                | 900.3                          | 871.9                             | 6.3         | 97                |
|  | 10                                | 90.3                           | 82.8                              | 37.1        | 92                |
|  | 10                                | 8.3                            | 7.6                               | 22.9        | 91                |
| IL-8   | 10                                | 900.3                          | 774.4                             | 1.8         | 86                |
|  | 10                                | 90.3                           | 83.4                              | 13.5        | 92                |
|  | 10                                | 8.3                            | 7.9                               | 12.6        | 95                |
| IL-11  | 10                                | 900.3                          | 901.8                             | 1.5         | 100               |
|  | 10                                | 90.3                           | 90.7                              | 19.6        | 100               |
|  | 10                                | 8.3                            | 9.3                               | 36.8        | 112               |
| IL-12  | 10                                | 900.3                          | 770.9                             | 7.3         | 86                |





| <b>TNF-<math>\alpha</math> Assay Specificity Test in Spiked Plasma (n=3 cartridges, 3 instruments per level)</b> |                                   |                                |                                   |             |                   |
|--|-----------------------------------|--------------------------------|-----------------------------------|-------------|-------------------|
| <b>Substance</b>   | <b>[Test Substance]<br/>ng/mL</b> | <b>Target<br/>[TNFa] pg/mL</b> | <b>Recovered<br/>[TNFa] pg/mL</b> | <b>CV %</b> | <b>% Recovery</b> |
|  | 10                                | 90.3                           | 77.4                              | 15.8        | 86                |
|  | 10                                | 8.3                            | 7.9                               | 56.7        | 96                |
| CNTF   | 10                                | 900.3                          | 920.1                             | 6.0         | 102               |
|  | 10                                | 90.3                           | 82.5                              | 9.7         | 91                |
|  | 10                                | 8.3                            | 8.7                               | 18.9        | 105               |
| G-CSF  | 10                                | 900.3                          | 1052.6                            | 3.7         | 117               |
|  | 10                                | 90.3                           | 95.6                              | 20.7        | 106               |
|  | 10                                | 8.3                            | 9.1                               | 9.6         | 110               |
| sgp130   | 1000                              | 900.3                          | 891.3                             | 16.8        | 99                |
|  | 1000                              | 90.3                           | 93.8                              | 9.1         | 104               |
|  | 1000                              | 8.3                            | 10.1                              | 25.1        | 122               |
| LIF R  | 50                                | 900.3                          | 781.5                             | 20.7        | 87                |
|  | 50                                | 90.3                           | 87.3                              | 15.2        | 97                |
|  | 50                                | 8.3                            | 9.1                               | 12.1        | 110               |
| OSM  | 10                                | 900.3                          | 862.1                             | 10.6        | 96                |
|  | 10                                | 90.3                           | 85.2                              | 23.8        | 94                |
|  | 10                                | 8.3                            | 7.4                               | 54.1        | 89                |
| TNF- $\beta$   | 10                                | 900.3                          | 804.0                             | 24.7        | 89                |
|  | 10                                | 90.3                           | 90.7                              | 16.4        | 100               |
|  | 10                                | 8.3                            | 7.7                               | 32.3        | 92                |
| IL-1 $\beta$   | 10                                | 900.3                          | 900.0                             | 17.3        | 100               |
|  | 10                                | 90.3                           | 83.1                              | 16.6        | 92                |
|  | 10                                | 8.3                            | 8.3                               | 33.1        | 101               |
| sTNF RI  | 10                                | 900.3                          | 833.0                             | 21.8        | 93                |
|  | 10                                | 90.3                           | 86.4                              | 19.5        | 96                |
|  | 10                                | 8.3                            | 6.7                               | 21.6        | 80                |
| sTNF RII   | 10                                | 900.3                          | 801.3                             | 8.9         | 89                |
|  | 10                                | 90.3                           | 93.6                              | 3.0         | 104               |
|  | 10                                | 8.3                            | 8.2                               | 14.2        | 99                |

| <b>CRP Assay Specificity Test in Assay Buffer (n=3 cartridges, 3 instruments per level)</b> |                                   |                               |                                  |             |                   |
|---|-----------------------------------|-------------------------------|----------------------------------|-------------|-------------------|
| <b>Substance</b>  | <b>[Test Substance]<br/>ng/mL</b> | <b>Target<br/>[CRP] ug/ml</b> | <b>Recovered<br/>[CRP] ug/ml</b> | <b>CV %</b> | <b>% Recovery</b> |
| Control   | 0                                 | 50                            | 53.0                             | 16          | 106               |
|   | 0                                 | 10                            | 8.1                              | 34          | 81                |
|   | 0                                 | 0.75                          | 0.7                              | 13          | 91                |
| Pentraxin-2/SAP   | 30                                | 50                            | 49.2                             | 19          | 98                |
|   | 30                                | 10                            | 8.9                              | 9           | 89                |
|   | 30                                | 0.75                          | 0.8                              | 4           | 102               |
| Pentraxin-3/TSG-14  | 10                                | 50                            | 40.6                             | 7           | 81                |
|   | 10                                | 10                            | 8.2                              | 14          | 82                |
|   | 10                                | 0.75                          | 0.7                              | 5           | 100               |



## 8. Linearity

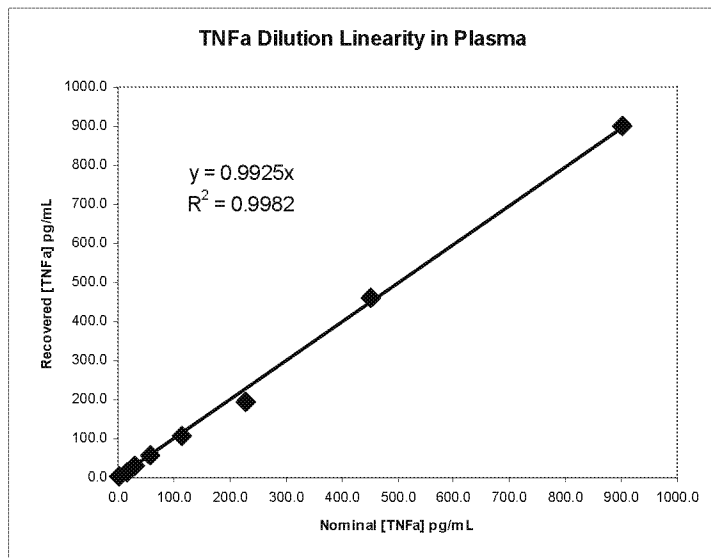
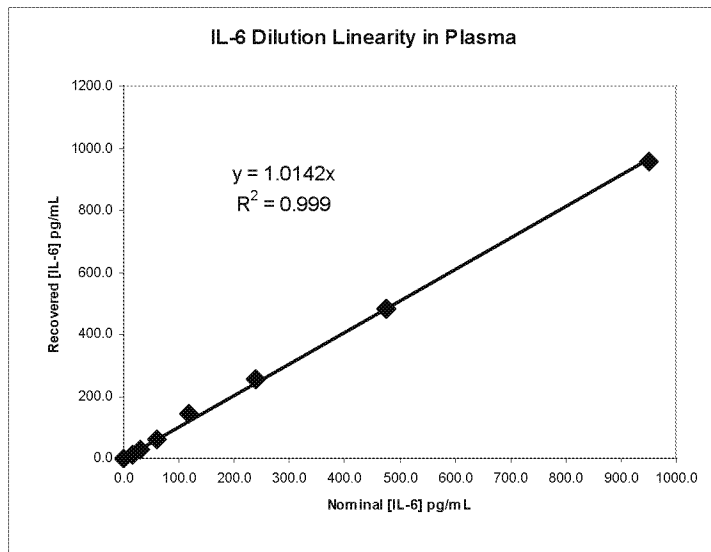
A plasma sample with low endogenous analyte levels was spiked with known levels of IL-6, TNF- $\alpha$ , and CRP then diluted serially with the unspiked plasma. All assays showed an appropriate linear dilution response across the dilution range (500 – 2000-fold). Data are tabulated and graphed below.

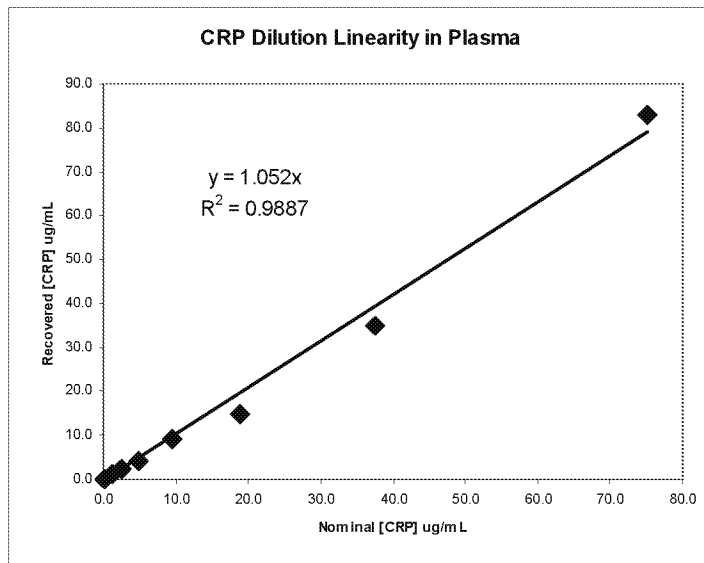
### **Dilution Linearity in Plasma, Multiplexed Assays (n=3 cartridges, 3 instruments per level)**

| <b>IL-6</b>                |                         |                          |             |                   |
|----------------------------|-------------------------|--------------------------|-------------|-------------------|
| <b>Spiked [IL-6] pg/mL</b> | <b>[Expected] pg/ml</b> | <b>[Recovered] pg/mL</b> | <b>CV %</b> | <b>% Recovery</b> |
| 950                        | 950.5                   | 958.1                    | 7           | 101               |
|                            | 475.5                   | 480.9                    | 11          | 101               |
|                            | 238.0                   | 256.1                    | 18          | 108               |
|                            | 119.2                   | 143.9                    | 25          | 121               |
|                            | 59.8                    | 62.3                     | 3           | 104               |
|                            | 30.1                    | 28.3                     | 23          | 94                |
|                            | 15.3                    | 13.3                     | 34          | 87                |
|                            | 0.5                     | 0.5                      | 88          | 100               |

| <b>TNF-<math>\alpha</math></b> |                         |                          |             |                   |
|--------------------------------|-------------------------|--------------------------|-------------|-------------------|
| <b>Spiked [TNFa] pg/mL</b>     | <b>[Expected] pg/ml</b> | <b>[Recovered] pg/mL</b> | <b>CV %</b> | <b>% Recovery</b> |
| 900                            | 902.7                   | 899.2                    | 11          | 100               |
|                                | 452.7                   | 461.5                    | 9           | 102               |
|                                | 227.7                   | 194.6                    | 6           | 85                |
|                                | 115.2                   | 105.0                    | 11          | 91                |
|                                | 59.0                    | 56.1                     | 2           | 95                |
|                                | 30.9                    | 30.6                     | 4           | 99                |
|                                | 16.8                    | 14.9                     | 26          | 89                |
|                                | 2.7                     | 2.7                      | 14          | 100               |

| <b>CRP</b>                |                         |                          |             |                   |
|---------------------------|-------------------------|--------------------------|-------------|-------------------|
| <b>Spiked [CRP] ug/mL</b> | <b>[Expected] ug/ml</b> | <b>[Recovered] ug/mL</b> | <b>CV %</b> | <b>% Recovery</b> |
| 75                        | 75.1                    | 82.8                     | 34          | 110               |
|                           | 37.6                    | 35.0                     | 0           | 93                |
|                           | 18.8                    | 14.7                     | 10          | 78                |
|                           | 9.5                     | 9.1                      | 12          | 96                |
|                           | 4.8                     | 4.1                      | 8           | 85                |
|                           | 2.4                     | 2.4                      | 7           | 98                |
|                           | 1.3                     | 1.3                      | 15          | 102               |
|                           | 0.1                     | 0.1                      | 29          | 100               |





## 9. Matrix Effects

Plasma or serum containing various potentially interfering factors or substances were spiked with known levels of analyte and the resulting recovery of the spiked analyte calculated after correction for endogenous analyte. None of the assays showed interference from icteric, hemolyzed, lipemic, or rheumatoid factor-positive samples as shown in the tables below

**NORMAL SERUM Sample: PromedDx 10739123 (n=3 cartridges, 3 instruments per level)**

| Spiked [IL-6] pg/mL | Recovered [IL-6] pg/mL | CV % | Minus Endogenous | % Recovery |
|---------------------|------------------------|------|------------------|------------|
| 1000                | 1019.1                 | 14   | 1015.82          | 102        |
| 250                 | 224.9                  | 4    | 221.58           | 89         |
| 50                  | 47.7                   | 14   | 44.42            | 89         |
| 25                  | 25.3                   | 6    | 22.01            | 88         |
| 10                  | 12.6                   | 9    | 9.29             | 93         |
| 0                   | 3.3                    | 43   | 0.00             |            |
| Spiked [TNFa] pg/mL | Recovered [TNFa] pg/mL | CV % | Minus Endogenous | % Recovery |
| 1000                | 1019.1                 | 14   | 1014.7           | 101        |
| 250                 | 224.9                  | 4    | 220.5            | 88         |
| 50                  | 47.7                   | 14   | 43.3             | 87         |
| 25                  | 25.3                   | 6    | 20.9             | 84         |
| 10                  | 12.6                   | 9    | 8.2              | 82         |
| 0                   | 4.4                    | 60   | 0.0              |            |
| Spiked [CRP] ug/mL  | Recovered [CRP] ug/mL  | CV % | Minus Endogenous | % Recovery |
| 100                 | 107.4                  | 11   | 107.3            | 107        |
| 50                  | 49.3                   | 13   | 49.3             | 99         |
| 25                  | 25.0                   | 23   | 24.9             | 100        |
| 10                  | 9.6                    | 41   | 9.5              | 95         |
| 5                   | 5.9                    | 17   | 5.8              | 116        |
| 0                   | 0.1                    | 12   | 0.0              |            |

**LIPEMIC SERUM Sample: Vital Products SFB8315 (n=3 cartridges, 3 instruments per level)**

| <b>Spiked [IL-6] pg/mL</b> | <b>Recovered [IL-6] pg/mL</b> | <b>CV %</b> | <b>Minus Endogenous</b> | <b>% Recovery</b> |
|----------------------------|-------------------------------|-------------|-------------------------|-------------------|
| 1000                       | 872.5                         | 15          | 868.8                   | 87                |
| 250                        | 214.1                         | 4           | 210.4                   | 84                |
| 50                         | 47.8                          | 15          | 44.1                    | 88                |
| 25                         | 24.5                          | 6           | 20.8                    | 83                |
| 10                         | 14.4                          | 19          | 10.7                    | 107               |
| 0                          | 3.7                           | 12          | 0.0                     |                   |
| <b>Spiked [TNFa] pg/mL</b> | <b>Recovered [TNFa] pg/mL</b> | <b>CV %</b> | <b>Minus Endogenous</b> | <b>% Recovery</b> |
| 1000                       | 965.0                         | 17          | 962.8                   | 96                |
| 250                        | 230.8                         | 15          | 228.6                   | 91                |
| 50                         | 56.6                          | 40          | 54.4                    | 109               |
| 25                         | 25.4                          | 13          | 23.2                    | 93                |
| 10                         | 14.8                          | 14          | 12.6                    | 126               |
| 0                          | 2.2                           | 32          | 0.0                     |                   |
| <b>Spiked [CRP] ug/mL</b>  | <b>Recovered [CRP] ug/mL</b>  | <b>CV %</b> | <b>Minus Endogenous</b> | <b>% Recovery</b> |
| 100                        | 119.4                         | 36          | 119.1                   | 119               |
| 50                         | 54.2                          | 40          | 53.9                    | 108               |
| 25                         | 24.4                          | 25          | 24.1                    | 96                |
| 10                         | 10.4                          | 9           | 10.1                    | 101               |
| 5                          | 5.8                           | 15          | 5.6                     | 111               |
| 0                          | 0.2                           | 12          | 0.0                     |                   |

**HEMOLYZED PLASMA Sample: Stanford W070509118560 (n=3 cartridges, 3 instruments per level)**

| <b>Spiked [IL-6] pg/mL</b> | <b>Recovered [IL-6] pg/mL</b> | <b>CV %</b> | <b>Minus Endogenous</b> | <b>% Recovery</b> |
|----------------------------|-------------------------------|-------------|-------------------------|-------------------|
| 1000                       | 1010.9                        | 10          | 1010.0                  | 101               |
| 250                        | 274.6                         | 13          | 273.7                   | 109               |
| 50                         | 51.6                          | 2           | 50.7                    | 101               |
| 25                         | 26.8                          | 11          | 25.9                    | 104               |
| 10                         | 10.5                          | 12          | 9.6                     | 96                |
| 0                          | 0.9                           | 41          | 0.0                     |                   |
| <b>Spiked [TNFa] pg/mL</b> | <b>Recovered [TNFa] pg/mL</b> | <b>CV %</b> | <b>Minus Endogenous</b> | <b>% Recovery</b> |
| 1000                       | 898.7                         | 14          | 895.1                   | 90                |
| 250                        | 223.5                         | 12          | 219.9                   | 88                |
| 50                         | 44.2                          | 11          | 40.6                    | 81                |
| 25                         | 27.7                          | 23          | 24.1                    | 96                |
| 10                         | 12.0                          | 23          | 8.4                     | 84                |
| 0                          | 3.6                           | 14          | 0.0                     |                   |
| <b>Spiked [CRP] ug/mL</b>  | <b>Recovered [CRP] ug/mL</b>  | <b>CV %</b> | <b>Minus Endogenous</b> | <b>% Recovery</b> |
| 100                        | 119.6                         | 10          | 119.5                   | 119               |
| 50                         | 54.0                          | 10          | 53.9                    | 108               |
| 25                         | 22.5                          | 14          | 22.4                    | 90                |
| 10                         | 11.6                          | 3           | 11.5                    | 115               |
| 5                          | 5.6                           | 11          | 5.5                     | 110               |
| 0                          | 0.1                           | 4           | 0.0                     |                   |

**ICTERIC SERUM Sample: PromedDx 10739123 (n=3 cartridges, 3 instruments per level)**

| <b>Spiked [IL-6] pg/mL</b> | <b>Recovered [IL-6] pg/mL</b> | <b>CV %</b> | <b>Minus Endogenous</b> | <b>% Recovery</b> |
|----------------------------|-------------------------------|-------------|-------------------------|-------------------|
| 1000                       | 986.0                         | 9           | 983.4                   | 98                |
| 250                        | 282.4                         | 12          | 279.7                   | 112               |
| 50                         | 55.8                          | 10          | 53.2                    | 106               |
| 25                         | 28.1                          | 7           | 25.4                    | 102               |
| 10                         | 11.8                          | 16          | 9.2                     | 92                |
| 0                          | 2.6                           | 53          | 0.0                     |                   |
| <b>Spiked [TNFa] pg/mL</b> | <b>Recovered [TNFa] pg/mL</b> | <b>CV %</b> | <b>Minus Endogenous</b> | <b>% Recovery</b> |
| 1000                       | 969.8                         | 5           | 967.4                   | 97                |
| 250                        | 219.6                         | 22          | 217.2                   | 87                |
| 50                         | 45.0                          | 11          | 42.6                    | 85                |
| 25                         | 24.5                          | 5           | 22.1                    | 88                |
| 10                         | 10.6                          | 22          | 8.2                     | 82                |
| 0                          | 2.4                           | 17          | 0.0                     |                   |
| <b>Spiked [CRP] ug/mL</b>  | <b>Recovered [CRP] ug/mL</b>  | <b>CV %</b> | <b>Minus Endogenous</b> | <b>% Recovery</b> |
| 100                        | 109.5                         | 8           | 108.4                   | 108               |
| 50                         | 41.7                          | 80          | 40.6                    | 81                |
| 25                         | 29.6                          | 14          | 28.4                    | 114               |
| 10                         | 10.1                          | 11          | 9.0                     | 90                |
| 5                          | 6.4                           | 19          | 5.3                     | 106               |
| 0                          | 1.1                           | 3           | 0.0                     |                   |

**RHEUMATOID FACTOR POSITIVE SERUM Sample: Vital Products SFB7884 (n=3 cartridges, 3 instruments per level)**

| <b>Spiked [IL-6] pg/mL</b> | <b>Recovered [IL-6] pg/mL</b> | <b>CV %</b> | <b>Minus Endogenous</b> | <b>% Recovery</b> |
|----------------------------|-------------------------------|-------------|-------------------------|-------------------|
| 1000                       | 1118.0                        | 10          | 1097.9                  | 110               |
| 250                        | 286.9                         | 9           | 266.7                   | 107               |
| 50                         | 77.7                          | 13          | 57.6                    | 115               |
| 25                         | 46.3                          | 12          | 26.2                    | 105               |
| 10                         | 30.4                          | 6           | 10.2                    | 102               |
| 0                          | 20.1                          | 6           | 0.0                     |                   |
| <b>Spiked [TNFa] pg/mL</b> | <b>Recovered [TNFa] pg/mL</b> | <b>CV %</b> | <b>Minus Endogenous</b> | <b>% Recovery</b> |
| 1000                       | 1116.4                        | 11          | 1112.3                  | 111               |
| 250                        | 228.9                         | 5           | 224.8                   | 90                |
| 50                         | 48.0                          | 13          | 43.9                    | 88                |
| 25                         | 24.2                          | 13          | 20.1                    | 80                |
| 10                         | 14.0                          | 20          | 9.9                     | 99                |
| 0                          | 4.1                           | 27          | 0.0                     |                   |
| <b>Spiked [CRP] ug/mL</b>  | <b>Recovered [CRP] ug/mL</b>  | <b>CV %</b> | <b>Minus Endogenous</b> | <b>% Recovery</b> |
| 100                        | 110.9                         | 18          | 105.8                   | 106               |
| 50                         | 49.1                          | 17          | 44.0                    | 88                |
| 25                         | 34.2                          | 29          | 29.0                    | 116               |
| 10                         | 15.5                          | 9           | 10.3                    | 103               |
| 5                          | 10.9                          | 11          | 5.7                     | 114               |
| 0                          | 5.2                           | 28          | 0.0                     |                   |



## 10. Stability

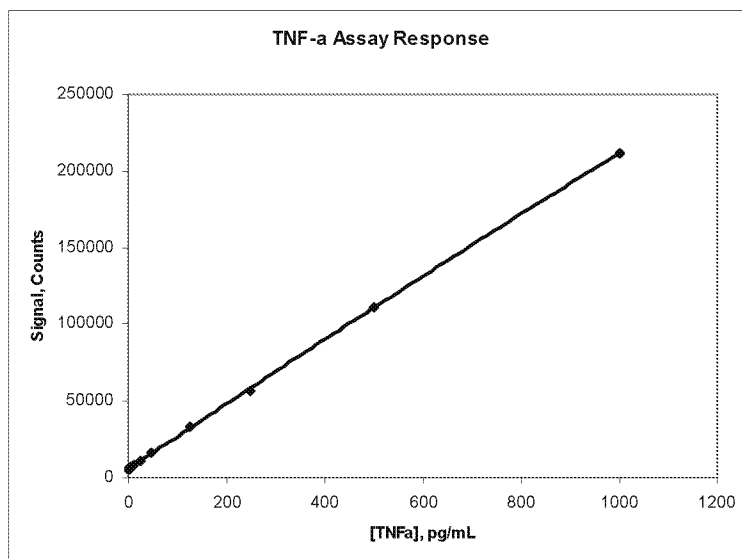
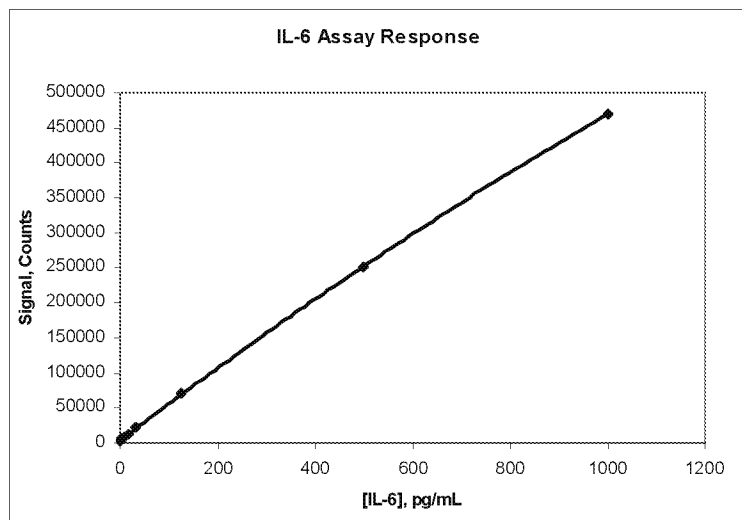
The stability of component reagents for the present assays has been studied individually in lots made previous to the present study. The capture surfaces were stable for over 12 months, and the detection conjugates for at least six months. Stability of the integrated cartridges used for this validation report stored at 4C is being monitored and an updated report will include this data. Cartridges are initially assigned an expiry date of three months post manufacture.

## Conclusions:

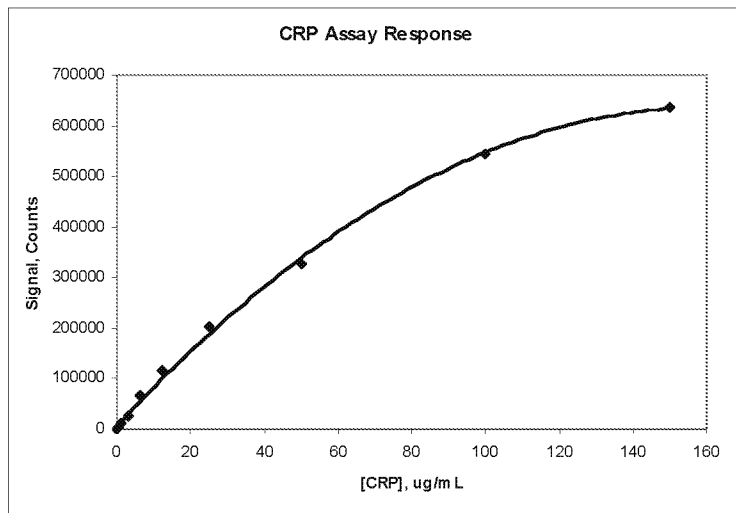
The Theranos IL-6, TNF- $\alpha$ , CRP assay multiplex has been shown to give accurate and precise results for three independently calibrated cartridge lots and all the many instruments used. Assay calibration has been established using WHO or other standard materials. Lower and upper levels of quantitation have been established. The assays are specific for their respective analytes when tested against potential cross reactants and are not interfered with by agents that may cause problems in immunoassays. Dilution linearity is satisfactory for all the assays. Assay cartridge stability studies are underway.



## Appendix A









## Appendix B

### Comparison of Theranos Systems TNFa Calibration to Other Available Commercial Methods

Plasma samples were spiked with WHO TNF-a Standard (NIBSC code 88/786) and run in Theranos Systems and in R&D Quantikine High Sensitivity Human TNF- $\alpha$  ELISA (catalogue # HSTA00D). The results are shown below.

#### THERANOS SYSTEMS Recovery of TNFa WHO Standard Spiked in Plasma

| Nominal Spike |              | 1pg/mL = 0.0465 IU/mL |                  |             |            |
|---------------|--------------|-----------------------|------------------|-------------|------------|
| [TNFa] IU/ml  | [TNFa] pg/ml | Calc. pg/mL           | Minus Endogenous | Calc. IU/mL | % Recovery |
| 0             | 0            | 5.2                   | 0.0              |             |            |
| 0.1           | 2.5          | 8.1                   | 2.9              | 0.1         | 118        |
| 0.2           | 5            | 11.5                  | 6.3              | 0.3         | 126        |
| 0.5           | 10           | 14.9                  | 9.7              | 0.5         | 97         |
| 1.2           | 25           | 35.9                  | 30.8             | 1.4         | 123        |
| 2.3           | 50           | 57.6                  | 52.4             | 2.4         | 105        |
| 11.6          | 250          | 217.6                 | 212.5            | 9.9         | 85         |
| 46.5          | 1000         | 1120.6                | 1115.4           | 51.9        | 112        |

#### R&D QUANTI KINE HS ELISA Recovery of TNFa WHO Standard Spiked in Plasma

| Nominal Spike |              | 1pg/mL = 0.0465 IU/mL |                  |             |            |
|---------------|--------------|-----------------------|------------------|-------------|------------|
| [TNFa] IU/ml  | [TNFa] pg/ml | Calc. pg/mL           | Minus Endogenous | Calc. IU/mL | % Recovery |
| 0             | 0            | 0.2                   | 0.0              |             |            |
| 0.1           | 2.5          | 1.0                   | 0.8              | 0.04        | 32         |
| 0.2           | 5            | 1.8                   | 1.6              | 0.07        | 32         |
| 0.5           | 10           | 3.2                   | 3.0              | 0.14        | 30         |
| 1.2           | 25           | 7.3                   | 7.1              | 0.3         | 28         |
| 2.3           | 50           | 15.0                  | 14.8             | 0.7         | 30         |
| 11.6          | 250          | 83.6                  | 83.4             | 3.9         | 33         |
| 46.5          | 1000         | 308.0                 | 307.7            | 14.3        | 31         |

